

Research Article

p53 Gene Codon 72 Polymorphisms among Cervical Carcinoma Patients

*Polimorfisme Gen p53 Kodon 72 pada Penderita Karsinoma Serviks yang Dirawat*Rustham Basyar¹, Agustria Z Saleh¹, Irawan Sastradinata¹, Yuwono²¹Department of Obstetrics and Gynecology²Department of Microbiology

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Abstract

Objective: To identify the association between p53 gene codon 72 polymorphism and cervical carcinoma.**Method:** An analytic observational study with case-control design, from November 2013 until March 2014 in the Department of Obstetrics and Gynecology and Microbiology Laboratory Faculty of Medicine, Sriwijaya University, Dr. Moh. Hoesin Hospital Palembang.**Result:** In total there are 100 samples analyzed consisting of 50 subjects and 50 control groups. Genotype distribution in subject group are 54% Arg/Arg, 42% Pro/Arg and 4% Pro/Pro, and in control group are 36% Arg/Arg, 46% Pro/Arg and 18% Pro/Pro. Arg/Arg genotype is at risk of cervical carcinoma 6.7 times higher compared with Pro/Pro genotype ($p=0.013$; OR 6.75; 95% CI 1.34-34.94). Arg allele in the p53 gene codon 72 increase the risk of cervical carcinoma 2.6 times more than Pro allele.**Conclusion:** Proline mutation to Arginine in gene p53 P72R is one of the risk factor for cervical carcinoma.

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Keywords: arginine, cervical carcinoma, gene p53 codon 72, polymorphism, proline

Abstrak

Tujuan: Mengetahui hubungan antara polimorfisme gen p53 kodon 72 dan karsinoma serviks.**Metode:** Penelitian observasional analitik dengan desain studi kasus-kontrol. Dilakukan dari November 2013 sampai dengan Maret 2014 bertempat di Departemen Obstetri dan Ginekologi Rumah Sakit Dr. Mohammad Hoesin Palembang dan Laboratorium Mikrobiologi Rumah Sakit Dr. Moh. Hoesin Palembang.**Hasil:** Penelitian diikuti oleh 100 subjek yang memenuhi kriteria inklusi, masing-masing 50 subjek pada kelompok kasus dan kontrol. Distribusi genotipe gen p53 kodon 72 pada kelompok kasus adalah Arg/Arg 54%, Pro/Arg 42% dan Pro/Pro 4% sedangkan pada kelompok kontrol Arg/Arg 36%, Pro/Arg 46% dan Pro/Pro 18%. Genotip Arg/Arg memiliki risiko 6,7 kali lebih besar untuk terjadi karsinoma serviks dibandingkan dengan genotip Pro/Pro. Adanya alel Arg pada gen p53 kodon 72 meningkatkan risiko terjadinya karsinoma serviks 2,6 kali lebih besar dibandingkan alel Pro.**Kesimpulan:** Mutasi Prolin menjadi Arginin pada polimorfisme gen p53 P72R merupakan faktor risiko terjadinya karsinoma serviks.

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Kata kunci: arginin, gen p53 kodon 72, karsinoma serviks, polimorfisme, prolin**Correspondence:** Rustham Basyar. Obstetrics and Gynecology Department. Faculty of Medicine, Sriwijaya University Palembang.
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INTRODUCTION

Cervical carcinoma is the most common gynecological malignancy and the second most common malignancies in women worldwide. Data from the Department of Anatomical Pathology Faculty Of Medicine, Sriwijaya University RSMH Palembang also places cervical carcinoma as the second most common malignancy in women, after breast malignancy.¹⁻⁴

Although the role of HPV in cervical cancer carcinogenesis has been proven, only a few of HPV infection will develop into cervical carcinoma. This suggests possibility of other influences that might be involved in the development of cervical carcinoma, one

of them is genetic factor. Mutation of the p53 gene is one of the genetic variations associated with the occurrence of malignancy.⁵⁻⁷

P53 gene is a tumor suppressor gene that plays a role in response to stress in order to maintain genomic stability, responds to DNA damage, hypoxia, metabolic stress and activation of oncogenes. Single nucleotide polymorphisms (SNPs) of p53 gene codon 72 will produce two types of alleles, namely Arginine (p53-R72) or Proline (p53-P72) at the structure of the amino acid at codon 72. According to Storey, women who are homozygous for Arginine at codon 72 of p53 gene are seven times more likely to be exposed to cervical cancer.^{6,9-11}

Comparative sequence analysis of non-human primates demonstrated that p53-P72 is the wild type, although the number of p53-R72 population is quite enough (>50%). The frequency of homozygous Arginine in certain ethnicity is between 12.4% and 53.4%. Beckman and his colleagues were the first to demonstrate the difference in allele frequency distribution between the R72 and P72 in Nigerian population (black Africans) and Sweden (Europe West), amounting 17 percents and 63 percents of the population.^{6,9,11-12}

Variation of the allele frequency in some ethnicities may cause differences in research result about relationship between p53 codon 72 polymorphism and cervical carcinoma in several countries. Research in Sweden, Chile, Peru, China and India supported these findings.^{5,8,13-16} However, studies in Netherlands, USA, Poland, Italy, Korea, Japan and Thailand are contradictory.¹⁷⁻²⁶ In Palembang, there is no data about codon 72 allele of the p53 gene and its association with carcinoma of the cervix, so it is necessary to identify the association between p53 codon 72 polymorphism and cervical carcinoma in Palembang population.

METHOD

This is an analytical observational research with case control study design. This study is conducted at the Department of Obstetrics and Gynecology and Microbiology Laboratory of Faculty of Medicine Sriwijaya University in Dr. Mohammad Hoesin Hospital Palembang, which started from November 1st 2013 until March 31st 2014.

Populations of this study are patients who are diagnosed with cervical carcinoma who came to the outpatient clinic and any hospitalized patient in obstetric and gynecology department of RSMH Palembang. Sampling is done by quota sampling technique, until total subjects in the experimental group and control group reach 50 patients.

The inclusion criteria for the experimental group are patients who are diagnosed with cervical carcinoma, which is proved by histopathology result and patient's willingness to join the research by signing the informed consent form. For the control group, inclusion criteria are patients who came to gynecology outpatient clinic or who has been hospitalized in in-patient installation of obstetric and gynecology department of RSMH Palembang who is not diagnosed or suspected to have neoplasm or another

malignancy diseases, no precancerous lesion Pap Smear examination or suspected to have cervical carcinoma and patient's willingness to join the research by signing the informed consent form.

Exclusion criteria for case group are patients who have been diagnosed or suspected to have other malignancy or if patient are not willing to join the research. For the control group, criteria were precancerous lesion in Pap's Smear result or suspected cervical carcinoma patients who refuse to join the research.

RESULT

Table 1 shows the socio-demographic and clinical characteristic of research subjects.

Table 1. Socio-demographic and Clinical Characteristic.

Characteristic	Case	Control	p
Median age	49 ± 8.8	38.8 ± 9.5	< 0.0001
Median BMI	23.22 ± 4.90	21.64 ± 31.08	0.058
Domicile			
Urban area	31	41	0.03
Rural area	19	9	
Ethnic group			
Sumatera	33	42	0.038
Non-Sumatera	17	8	
Education			
Uneducated	5	0	< 0.0001
Elementary	14	1	
Junior high	14	6	
Senior high	16	22	
Bachelor	1	21	
Occupation			
Housewife	38	22	< 0.0001
Civil servant	1	27	
Others	11	1	
Smoking history			
Yes	2	2	1.00
No	48	48	
Contraception history Oral			
Yes	9	4	0.23
No	41	46	
First sexual activity			
< 20 years old	33	8	< 0.001
≥ 20 years old	17	42	
Marriage history			
1 time	6	2	0.27
> 1 time	44	48	
Parity total			
≥ 7	8	0	< 0.001
< 7	42	50	

The youngest age in this research is 19 years old and the oldest subject is 73 years old. There is a significant difference in median age, domicile distribution, ethnic group and occupation between the case and control group.

Age of first sexual activity and total parity are important clinical risk factors for cervical carcinoma. However smoking, oral contraception, and marriage status showed no significant correlation with the incidence of cervical carcinoma. The clinical characteristics of the research subjects are presented on the table above.

In this research gene p53 codon 72 is obtained through DNA extraction process and PCR-RFLP method from blood sample. Polymorphism in gene p53 codon 72 is recognized with BstUI enzyme. Figure 1 showing the PCR-RFLP result example. Genotype appearance of wild type Pro/Pro (1 band with fragment length 199 bp) is showed in subject number 46. Subject number 50 has mutant genotype Arg/Arg (2 bands with fragment length 113 bp and 86 bp). Subject number 47, 48 and 49 exhibit mutant heterozygote genotype appearance of Pro/Arg (3 bands with fragment lengths of 199 bp, 113 bp and 86 bp).

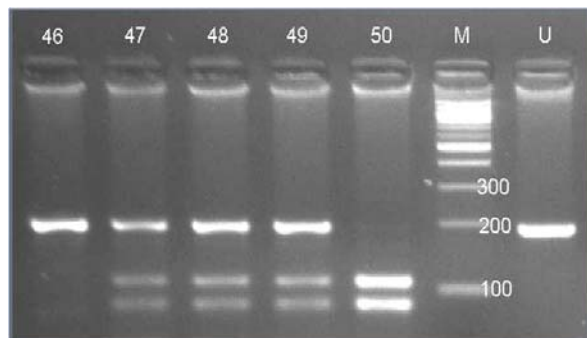


Figure 1. Example of PCR-RFLP Result Gene p53 Codon 72 with BstUI Restriction Enzyme.

Genotype distribution in case group is Arg/Arg 54%, Pro/Arg 42% and Pro/Pro 4%. The distribution of genotype in control group is Arg/Arg 36%, Pro/Arg 46% and Pro/Pro 18%. In general, the distribution of genotype Arg/Arg, Pro/Arg and Pro/Pro are 45%, 44% and 11% respectively. From the whole subjects, Arg allele percentage is 67% and Pro allele is 33%.

Statistic analysis with Chi square test is done to determine the correlation between polymorphism gene p53 codon 72 and cervical carcinoma. There is a significant increase in the risk of cervical carcinoma

for individual with homozygote mutant genotype (Arg/Arg) compared with individual with wild type genotype (Pro/Pro) on gene p53 codon 72 ($p=0.013$; OR 6.75; 95% CI 1.34-34.94). Allele analysis showed women who has Arginine allele in the p53 gene codon 72 is at risk of cervical cancer 2,6 times greater than women with Proline allele (OR 2.61; 95% CI 1.40-4.88).

Table 2. Correlation of Genotype p53 Gene Codon 72 and Carcinoma Cervix

		Case		Control		P
		N	%	N	%	
Genotype	Arg/Arg	27	54	18	36	0.013*
	Pro/Arg	21	42	23	46	0.097**
	Pro/Pro	2	4	9	18	Ref
Total		29	100	27	100	

DISCUSSION

Epidemiological data shows that most cervical carcinoma is found in young women. Median age of this finding is 52 years old with distribution peak at age of 35-39 years old and 60-64 years old.^{1,3} Concurrent with previous literature, median age of the case group (cervical carcinoma) in this research is 49.8 ± 8.8 years old.

In this research, there is a significant difference in domicile, education level, job and ethnic group between case group and control group. Whereas median body mass index (BMI) in this research subject found no differences between both groups. Socioeconomic factor is related with carcinoma cervix. The majority of carcinoma cervix patients came from low socioeconomic who has no access to routine gynecology examination.^{1,3} Reis et al discovered that individual with high education level has lower risk of cervical carcinoma (OR 0.18). They suggested that low education level is related with an increase of sexual activity on young age, numerous sexual partners and bad genital hygiene.²⁷

Statistically in this research there is no significant correlation between smoking history and cervical carcinoma. This result is different with meta-analysis about correlation between smoking and cervical carcinoma by Gandini et al. They concluded that active smokers are at increased risk of contracting cervical squamous cell carcinoma (RR 2.03; 95% CI 1.31-4.04), whereas risk among ex-smoker is relatively lower at 1.80 (95% CI 0.95-3.44).²⁸

Reproductive history is also affects the risk of cervical carcinoma. Analysis result in this research also shows significant correlation between parity ≥ 7 and cervical carcinoma ($p=0.006$). Women who has parity ≥ 7 times has 2 times the risk to have carcinoma cervix (OR 2.19; 95% CI 1.75-2.74).

Median age of first sexual activity in the case group in this research is 19.12 ± 3.98 years old. This median age is lower than the control group. Statistical analysis shows an increased risk of cervical carcinoma 10 times higher than women who have their first sexual activity before the age of 20 is 10.19 (95% CI 3.92-26.51).

Oral contraception combination pill usage can be a risk factor of cervical carcinoma. Research from Moreno et al show an odds ratio of 2.82 (95% CI 1.46-5.42) for contraception usage 5-9 years and 4.03 (95% CI 2.09-8.02) for usage ≥ 10 years.^{1,3,27,29,30} However in this research, there is no significant correlation between oral contraception and cervical carcinoma ($p=0.23$).

Comparative sequence analysis to primate except human shows that p53-R72 is a wild type, even though p53-R72 in population is varied ($>50\%$). Data from various ethnicities shows Arginine homozygous frequency is between 12.4% till 53.4%.^{6,9,27,31} Genotype distribution pattern and allele in this research is similar with Andersson's research in Sweden and Ojeda's in Chile; the only difference is the lower genotype distribution of wild type.^{5,13}

Chi square test done between gene genotype p53 codon 72 and cervical carcinoma concludes that there is an increasing risk of cervical carcinoma 6.7 times for individual with homozygote mutant genotype (Arg/Arg) compared with individual with wild type genotype (Pro/Pro) for gene p53 codon 72 (OR 6.75; 95% CI 1.34-34.94). Individuals with heterozygote mutant genotype (Pro/Arg) have no significant risk when compared with women with wild type genotype ($p=0.079$). Allele analysis shows significant result for allele Arg to p53 gene codon 72, which will increase the risk of carcinoma cervix 2.61 times higher than allele Pro ($p=0.02$; OR 2.61; 95% CI 1.40-4.88).

Some of supporting researches have various odds ratio (OR) result. Meta-analysis by Jee et al in 2004 shows that the odds ratio (OR) of women with cervical carcinoma with homozygote mutant (Arg/ Arg) compared with heterozygote mutant (Arg/ Pro) in general is 1.2 (1.1-1.3; $p<0.001$).³²

Some of the researches also relates polymorphism p53 codon 72 with prognosis and response to therapy. Individual with genotype R72 has a higher risk and better quality of life after receiving chemotherapy or radiation therapy. Similar results are also found in cancer of head and neck, breast and lung.⁹

CONCLUSION

Proline mutation to Arginine in gene p53 P72R is a risk factor for cervical carcinoma.

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